the recent study of Young-Min [29] who showed that Glc–Gal–PYD was predictive in bivariate, but not in multivariate analyses when CTX-II was included in the model. This lack of independent predictive value is likely to be due to the high correlation of Glc–Gal–PYD with CTX-II ($r = 0.61$, $p < 0.001$) and suggests that in early active RA, degradation of cartilage is closely linked to synovitis. Whether urinary Glc–Gal–PYD could be an independent predictor of progression in late RA or in patients receiving biological therapies remains to be determined.

Previously published cross-sectional studies found an increased urinary PYD/DPD ratio in patients with RA [46–49]. Our study, however, is the first showing that U-PYD/DPD ratio is an independent predictor of radiological progression. Both PYD and DPD are non-reducible cross-links of mature collagen molecules, and they are believed to be important factors for maintaining the structure of the collagen fibril network in the matrix of the various tissues, including bone and cartilage. In healthy tissues, the PYD/DPD ratio is highest in cartilage (ratio: 50), intermediate in synovial tissue and tendons (ratio: 15–16) and lowest in bone (ratio: 3.5) [50–52]. The tissue PYD/DPD ratio can be altered in RA tissue, with the latter showing a higher ratio than healthy synovium [23, 51]. In addition, a high tissue PYD/DPD ratio in bone caused by the overhydroxylation of Lys at the helical cross-linking sites in type I collagen has been observed in the hip fracture cases [53] and osteoporosis [54]. Thus, the PYD/DPD ratio may theoretically provide some indication of the type of articular tissue that is predominantly degraded in RA. In our study, this ratio, but not PYD and DPD separately, was associated with radiological progression of bone erosion and JSN independently of CTX-II, which is a specific marker of cartilage degradation and of Glc–Gal–PYD (a specific marker of synovial metabolism), suggesting indeed the added value of this parameter. One possibility is that this ratio partially reflects structural alterations of bone tissue matrix associated with increased bone fragility, as suggested by some ex vivo biochemical studies [53, 54].

We found that high BMI was correlated negatively with the progression of joint erosion and JSN and that patients with lower values (<18.5), defined as underweight, had a 4.8-fold (95% CI 1.1–20) higher risk than the patients with higher BMI (>25) who were defined as overweight. Previously published reports showed a body weight loss due to disease activity [55–58] in RA, although no significant correlation between BMI and inflammation markers was observed at baseline in our study (data not shown). Our results agree with studies published previously by Kaufmann [23], Westhoff [31] and van der Helm-van Mil [30] which showed that high BMI was protective against the radiological progression in early RA. It has been suggested that the relationships between BMI and joint damage are mediated in part by the adipocytokines secreted by fat tissues. Interestingly, we recently reported that increased serum levels of adiponectin—which is negatively associated with BMI—are associated with a greater overall joint destruction in patients with RA [59]. Using a bivariate analysis, we found that triglycerides, but not total cholesterol and its subfractions were negatively correlated with radiological progression. However, in the multiple variable model, triglycerides were not an independent predictors, possibly because of its positive association with BMI ($r = 0.29$, $p < 0.001$).

Previously published data showed that high initial radiographical damage evaluated with TSS or the Larsen score was associated with subsequent radiological progression [16, 17] and that the initial erosion score in particular has a predicting value for radiological prognosis [14, 18, 23]. These data were analyzed without biochemical markers of joint tissue turnover as the initial factors; however, we found that baseline radiological joint damage of the extent of JSN was strongly and independently predictive of biochemical markers of joint tissue turnover associated with progression.

We believe that the four independent predictors of radiological progression we identified in this study may reflect different and complementary information of the various pathophysiological processes involved in joint destruction. The baseline Sharp score provides an estimation of the amount of joint destruction that has occurred, on average, during 2.3 years of disease duration before the start of the follow-up. Urinary CTX-II is a dynamic indicator of the rate at which cartilage tissue will deteriorate during the course of the disease. The PYD/DPD ratio may be related to increased bone fragility, and the BMI may provide integrated information on contribution of adipose tissue metabolism to maintain joint tissues health. These four independent predictors were statistically selected using those patients with high disease activity who were participating in the control arm of the SAMURAI study and who had >6 tender joints (of 49 evaluated), >6 swollen joints (of 46 evaluated joints), ESR of >30 mm/h and CRP of >2 mg/dl. These predictors may therefore be beneficial for targeting new biological therapies to patients with rapid progression of joint destruction.

Although our study covered one of the largest ranges of predictive variables for the progression of joint damage ever investigated concomitantly in the same population, due to sample volume limitation we could not analyze a number of the biochemical markers that have been reported to be associated with joint damage in RA, including anti-CCP antibody, cartilage oligomeric matrix protein (COMP) [25, 26, 60], osteoprotegerin (OPG) and Receptor Activator of Nuclear Factor-kappa B Ligand (RANKL) [61]. Our

study included patients with RA within 5 years of disease duration, so it remains to be determined whether the same set of predictive factors will also perform similarly in patients with earlier RA. Furthermore, our study could not clarify the prognostic factors in the each type of DMARDs treatment nor whether CTX-II, the PYD/DPD ratio, the JSN score and BMI predict progression independent of the type of DMARDs treatment, since the dose, type and combination of DMARDs and/or immunosuppressants was varied and changed according to disease activity at the discretion of the treating physician in our study. However, our data could provide the prognostic values of CTX-II, PYD/DPD ratio, JSN score and BMI in the actual clinical practice of RA treatment.

In summary, among of a panel of 40 different variables, we identified baseline joint damage, urinary CTX-II, the PYD/DPD ratio and BMI as strong and independent factors of radiological progression in patients with RA receiving conventional DMARDs. If confirmed in other studies, this set of few variables may be useful to identify patients with RA who are at high risk for disease progression.

Acknowledgments The authors wish to thank Takahiro Kakehi, B.Sc., Tamiko Kuraishi, B.Sc., Yukiyasu Mariko, M.Sc. and Yuichi Kawata, B.Sc., for their valuable assistance with the design and analysis of the study and preparation of this manuscript. This work was supported by Chugai Pharmaceutical Co., Ltd., Tokyo, Japan.

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Ann Rheum Dis 2009 68: 654-657 originally published online June 2, 2008
doi: 10.1136/ard.2008.090068

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Laboratory and febrile features after joint surgery in patients with rheumatoid arthritis treated with tocilizumab

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Accepted 9 May 2008
Published Online First
2 June 2008

ABSTRACT

Objectives: To understand the acute phase responses to surgical intervention in patients with rheumatoid arthritis (RA) treated with the anti-interleukin (IL)6 receptor antibody, tocilizumab.

Methods: In a retrospective 1:1 pair-matched case-control study, 22 tocilizumab-treated RA cases and 22 cases treated with conventional disease-modifying antirheumatic drugs (DMARDs) and matched for type of surgery, age and sex were evaluated for body temperature every day, and blood C-reactive protein (CRP) levels and white blood cell (WBC), neutrophil and lymphocyte counts on days −1, 1, 3 and weeks 1 and 2 after joint surgery. Safety issues were also monitored.

Results: No complications of infection or delay of wound healing occurred in either patient group. Tocilizumab partially, but significantly, suppressed the increase in body temperature on postoperative days 1 and 2, compared with DMARDs (average (SD) maximum increase in temperature was 0.45 (0.1)°C in the tocilizumab group and 0.78 (0.1)°C in the DMARD group; $p<0.01$). Tocilizumab completely suppressed the increase in CRP after surgery, whereas all cases treated with DMARDs showed a significant increase of CRP at postoperative day 1 (5.5 (0.6) mg/dl; $p<0.001$). WBC, neutrophil and lymphocyte counts showed no remarkable change after surgery, and there was no significant difference in any cell counts between the patient groups.

Conclusions: Within this small number of cases, safe operations on patients were performed during tocilizumab treatment. Tocilizumab suppressed fever and increase of CRP after surgery, whereas there was no influence on the transition in number of leukocytes. This characteristic postoperative response should be considered during tocilizumab treatment.

Postoperative surgical site infections represent a serious functional and psychological disadvantage in the course of treatment against rheumatoid arthritis (RA), although the incidence of these infrequent complications in joint surgery performed in patients with RA is approximately 1.7% to 7.2%.[1–3] As it is necessary to detect postoperative infections as soon as possible, body temperature, level of C-reactive protein (CRP) and white blood cell (WBC) count, as well as local findings, are considered indicators of infection.

Previously published data show that the cytokine interleukin (IL)6 is upregulated by surgical trauma and involved in the febrile response.[2 3] The acute phase response might be diminished by blocking the synthesis of IL6 and has been reduced in genetically-modified animals that do not produce this mediator.[4 5] CRP is primarily produced in the liver in response to IL6, and CRP synthesis is enhanced synergistically by IL1β through induction of nuclear factor (NF)κB, p50 and p65.[6–8] In addition, IL6 positively regulates numbers of leukocytes and neutrophils, and inhibition of the IL6 receptor leads to a decrease in leukocytes.[9 10]

In recent years, use of tocilizumab, a humanised monoclonal antibody against IL6 receptor, has succeeded in achieving more effective suppression of disease activity of RA, compared to conventional disease-modifying antirheumatic drugs (DMARDs).[11 12] Based on the previous data, it is unknown whether tocilizumab also suppresses surgically-related and infection-related acute phase responses, thereby leading to possible difficulty in early diagnosis of postoperative infection due to lack of observed clinical signs and symptoms in patients treated with tocilizumab. Therefore, it is very important to understand the details of transition of data on blood tests and body temperature after joint surgery in patients with RA treated with tocilizumab. In the current study, we examined postoperative changes in body temperature and blood levels of CRP, WBCs, neutrophils and lymphocytes in patients with RA treated with tocilizumab or conventional DMARDs.

PATIENTS AND METHODS

Patients

A total of 22 joint surgeries in our hospital were performed on patients with RA treated with tocilizumab. In all cases, treatment with tocilizumab (8 mg/kg, every 4 weeks) was continued, and surgery was performed between the administrations of tocilizumab. The most recent infusion was performed 16.1 (9.5) days (range 3 to 27 days) (mean (SD)) before operation. There was no case of postponement of infusion. All surgeries were performed under general anaesthesia. Operations included shoulder arthroplasty (n = 1), total elbow arthroplasty (TEA) (n = 1), total hip arthroplasty (THA) (n = 1), total knee arthroplasty (TKA) (n = 9), total ankle arthroplasty (TAA) (n = 3), foot surgery (n = 5) and hand surgery (n = 2).

Retrospectively, we studied these 22 cases (tocilizumab group) and compared them to 22 operations in patients with RA who received non-biological medication (methotrexate: 6 cases; salazosulfapyridine: 10 cases, bucillamine: 3 cases, D-penicillamine: 1 case, prednisolone: 17 cases)

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and who underwent joint surgery. For matching of cases, patients were selected with a 1:1 pair-matched procedure, according to age (range ±5 years) and type and site of surgery.

Body temperature

Body temperature was measured at least three times daily (at 9.00 am, 2.00 pm and 7.00 pm) during hospitalisation. All measurements were performed for 5 min at the external ear canal on the same side. For analysis, we used the peak body temperature at 1 day before surgery, during 1 and 2 days after surgery, 1 week after surgery (week 1) and 2 weeks after surgery (week 2). Expected fever spikes after surgery in the tocilizumab and the DMARD groups were evaluated by change in body temperature, as compared before the operation.

Blood samples

Venous blood samples were obtained before surgery, 1 and 3 days after surgery, 1 week after surgery and 2 weeks after surgery. Samples were collected in EDTA tubes and analysed for WBC counts and segmentation of WBCs (neutrophils and lymphocytes). The change in WBCs, neutrophils and lymphocytes after surgery was evaluated by the percentage changes in these parameters, compared with preoperative values. CRP levels were measured in serum. In our hospital, the normal reference value for CRP was <0.2 mg/dl.

Statistical analysis

All data are expressed as mean (standard error of the mean (SEM)). Differences between the groups were assessed by a post hoc test using SPSS statistical analysis software (SPSS V. 15.0J; SPSS, Chicago, Illinois, USA). A p value of <0.05 was considered statistically significant.

RESULTS

Postoperative complications

No complications of superficial infections, deep infections, or delay in wound healing were observed in either group of cases.

Body temperature

As shown in table 1, there was no significant difference in preoperative body temperature between the tocilizumab and the DMARD groups. Both groups showed significant increases in body temperature, as compared with that of the preoperative day (fig 1). The expected increase in body temperature following surgery was significantly suppressed in the tocilizumab group (fig 1). Average maximum increase in body temperature was 0.78 (0.1)°C in the DMARD group and 0.45 (0.1)°C in the tocilizumab group.

CRP

Preoperative CRP levels in the tocilizumab group were negative in all cases except one, who showed 0.3 mg CRP/dl. All cases in the DMARD group showed positive CRP values, and the mean (SD) value was 3.1(0.6) mg/dl (table 1). After surgery, 18 of the 22 tocilizumab-treated cases showed no postoperative increase in CRP, whereas the remaining 4 cases showed an increase ranging from 0.1 to 1.0 mg/dl. In the DMARD group, all cases showed a significant increase (day 1: 5.5 (0.6) mg/dl; 1 week after surgery: 2.9 (0.5) mg/dl; 2 weeks after surgery: 2.2 (0.5) mg/dl).

WBC counts

As shown in table 1, there was no significant difference in preoperative numbers of WBCs, neutrophils, or lymphocytes between the tocilizumab and the DMARD groups. These cell numbers showed no remarkable changes after surgery in either group. Additionally, no significant difference between the groups was observed in these parameters after surgery. Therefore, cell numbers after surgery were not affected by tocilizumab treatment.

DISCUSSION

An important issue in anti-inflammatory cytokine therapy is the possible risk of infection, because a recent study showed that tumour necrosis factor (TNF)α antagonists increase the risk of infection for musculocutaneous lesions.[13] Reports on small studies involving 12 to 16 cases of joint surgery in patients with RA treated with TNFα antagonists, however, demonstrated no increase in the incidence of postoperative infection.[14 15]

Our hospital's experience with 22 cases of joint surgery in patients with RA treated with tocilizumab also showed no postoperative surgical site infections. Although IL6 knock-out (IL6KO) mice display significantly delayed cutaneous wound healing compared to wild type mice,[16 17] we observed no delay in postoperative wound healing in patients with RA treated with tocilizumab. Because the reported incidence of postoperative infection is around 1.7% to 7.2%,[1–3] the lack of statistical power due to the small number of patients in our study made it impossible to reach a conclusive result as to the influence of tocilizumab treatment on the risk of postoperative infection. However, our experience that there were no postoperative infections in either tocilizumab or DMARD groups suggests the safety of joint surgery in patients with RA during tocilizumab treatment.

High-level production of proinflammatory cytokines including IL6 in the inflamed synovium has a pathological role in systemic manifestations of RA, such as fatigue, fever and laboratory changes.[18] After surgical treatment, immune cells such as macrophages and neutrophils or other cells such as fibroblasts and endothelial cells are activated locally at the surgical site by destruction of tissues, resulting in increased local and serum levels of IL6.[2 19] Therefore, it is plausible that inhibition of IL6 suppresses the systemic inflammatory manifestations of RA and surgery. Indeed, the postoperative fever spike was partially, but significantly, suppressed by tocilizumab treatment, as compared with DMARD treatment in the present study.

IL6 stimulates hepatocytes to produce acute phase proteins such as CRP, fibrinogen, α1-anti-trypsin and serum amyloid A, and simultaneously suppresses albumin production. Previous reports have shown that the increase in CRP and serum amyloid A were normalised after tocilizumab treatment and the decrease in albumin was alleviated after tocilizumab treatment.[10–12 20] In the current study, the preoperative CRP values were normal (<0.2) in 21 out of 22 cases in the tocilizumab group. Furthermore, a postoperative increase in CRP was not observed in the tocilizumab group, whereas in the DMARD group the mean CRP level changed from a preoperative level of 3.1 mg/dl to a maximum level of 5.5 mg/dl 1 day after surgery. This finding clearly indicates that tocilizumab suppressed not only an RA-related increase of CRP, but also a surgery-related increase in CRP. Tocilizumab treatment also may suppress infection-related symptoms after surgery. Although no infection was observed in the patients of this study, we have experienced

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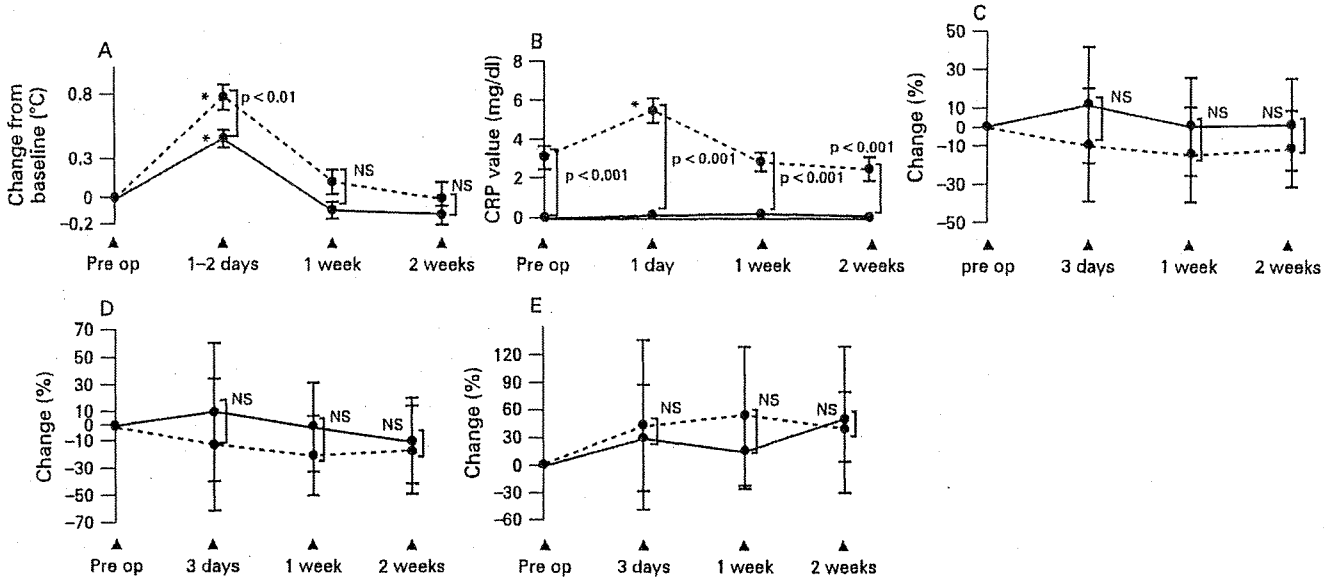

Figure 1 Changes in body temperature, C-reactive protein (CRP) levels and numbers of white blood cells (WBCs; neutrophils and lymphocytes) after surgery. Values are mean (SD). *Significant difference, as compared to values before surgery. NS, no significant difference between the two groups. Dotted lines: disease-modifying antirheumatic drug (DMARD) group. Unbroken lines: tocilizumab group. A. Graph shows the change in body temperature, as compared before surgery. p: p Value for the difference between the DMARD group and the tocilizumab group. B. Graph shows the absolute value of CRP level. p: p Value for the difference between the two groups. C. Graph shows the percentage change in total number of WBCs. D. Graph shows the percentage change in numbers of neutrophils. E. Graph shows the percentage change in numbers of lymphocytes.

some tocilizumab-treated patients with pneumonia without operation. As long as the infection was not severe, serum level of CRP hardly increased while the WBC count did increase. When the patients had severe pneumonia, they showed an increase in CRP level and WBC count (data not shown). This may be explained by the balance between the blood concentration of IL6 and tocilizumab, which competitively binds to IL6 receptor. Severe infection induces a high amount of IL6, which cannot be blocked with usual doses of tocilizumab, resulting in the increase in CRP. Similarly, if the surgical invasion is more severe, CRP would be expected to increase even under tocilizumab treatment. In addition, since the WBC count is less influenced by IL6 than CRP, an increase in WBC count could be a useful sign for possible infection. In the DMARD group, the level of CRP was restored to the basal level within 1 week after surgery, suggesting that surgery-induced inflammation resolves within 1 week in the absence of infection.

A dose-dependent reduction in the neutrophil count following treatment with tocilizumab was reported in patients with RA.[11 12] In the present study, preoperative absolute numbers of WBCs and neutrophils in the tocilizumab group were smaller, but not significantly smaller, than those in the DMARD group. By contrast, preoperative absolute numbers of lymphocytes in the tocilizumab group were larger, but not significantly larger, compared to the DMARD group. No significant postoperative changes in leukocytes, neutrophils, or lymphocytes were observed in either group, and additionally no significant differences between the two groups were seen at any time after surgery. These data suggest that regulation of the increase in leukocytes may depend on not only IL6 but also other cytokines such as granulocyte colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF).

Table 1 Characteristics of the cases before surgery

Parameter	No. treated with DMARDs	No. treated with tocilizumab	p Value
Body temperature (°C)	36.5 (0.3)	36.6 (0.2)	NS
CRP (mg/dl)	3.1 (0.6)	0.02 (0.02)	<0.001
WBCs/dl	8144 (3229)	7267 (2757)	NS
Neutrophils/ml	6716 (2973)	5424 (2545)	NS
Lymphocytes/ml	978 (413)	1384 (706)	NS
DAS28 (CRP) score	4.4 (0.9)	2.7 (0.7)	<0.001
Prednisolone (mg/day)	7.0 (5.1)	6.5 (5.1)	NS

Values are mean (SD). p Value is for the difference between the DMARD group and the tocilizumab group.
CRP, C-reactive protein; DAS28, 28-joint Disease Activity Score; DMARD, disease-modifying antirheumatic drug; NS, not significant; WBC, white blood cell.

Our data suggest the possibility that tocilizumab might mask the infection-induced increase in CRP and minimise the infection-induced increase in body temperature after orthopaedic surgery in patients with RA. In other words, a small postoperative increase in CRP in patients with RA may be an important sign of the occurrence of non-arthritic types of inflammation, such as postoperative infections. In cases of postoperative increases in CRP in tocilizumab recipients, signs of inflammation in other organs, such as the respiratory system, as well as signs of infection at the surgical site, should be checked. A WBC count may be helpful in knowing whether a concurrent infection is occurring during tocilizumab therapy. Furthermore, serum IL6 should be a good marker for the severity of inflammation because tocilizumab blocks not IL6 itself but the IL6 receptor.[4 5]

In conclusion, although the current study evaluated only 22 cases of orthopaedic surgery in patients with RA treated with tocilizumab and 22 cases of orthopaedic surgery in patients with RA treated with DMARDs, no complications of superficial or deep infection or delay in wound healing after orthopaedic surgery were observed. Additionally, we demonstrated that the increase in CRP was completely suppressed and the rise in body

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temperature was partially suppressed after joint surgery in tocilizumab-treated patients with RA, whereas tocilizumab had no significant influence on the number of leukocytes. Considering these characteristic postoperative responses in patients with RA treated with tocilizumab, we should carefully perform orthopaedic surgery on the patients.

Acknowledgements: We would like to thank Chugai Pharmaceuticals for providing tocilizumab.

Competing interests: NN and TM have served as consultants to and/or received honoraria from Chugai Pharmaceutical, the manufacture of tocilizumab.

Ethics approval: Ethics approval was obtained.

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International Immunopharmacology 9 (2009) 86–90

Contents lists available at ScienceDirect

International Immunopharmacology

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Differential influences of bucillamine and methotrexate on the generation of fibroblast-like cells from bone marrow CD34+ cells of rheumatoid arthritis patients

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ARTICLE INFO

Article history:
Received 17 March 2008
Received in revised form 16 September 2008
Accepted 8 October 2008

Keywords:
Rheumatoid arthritis
Bone marrow
CD34+ cells
TNF-α
Bucillamine
Methotrexate

ABSTRACT

We have recently demonstrated that bone marrow CD34+ cells from rheumatoid arthritis (RA) patients displayed abnormal capacities to respond to TNF-α and to differentiate into fibroblast-like cells producing MMP-1 (type B synoviocyte -like cells). The current study examined the effects of representative potent disease-modifying antirheumatic drugs, including bucillamine (BUC) and methotrexate (MTX) on the *in vitro* generation of fibroblast-like cells from RA bone marrow CD34+ cells. CD34+ cells purified from bone marrow specimens of 8 patients with active RA were cultured in the presence or absence of pharmacologically attainable concentrations of intramolecular disulfide form of bucillamine (BUC-ID, 3 μM), a major metabolite of BUC or MTX (20 nM). After incubation for 28 days, the generation of fibroblast-like cells was assessed under phase-contrast light microscopy and the concentrations of MMP-1 and VEGF in the culture supernatants were measured by ELISA. BUC-ID, but not MTX, significantly suppressed the generation of fibroblast-like cells from RA bone marrow CD34+ cells stimulated with SCF, GM-CSF and TNF-α ($p=0.024$ as determined by Wilcoxon signed rank test). Accordingly, BUC-ID, but not MTX, significantly suppressed the production of MMP-1 ($p=0.017$) and VEGF ($p=0.017$) by RA bone marrow CD34+ cells, without inhibition of β2-microglobulin production. These results demonstrate that BUC-ID, but not MTX, is a potent inhibitor of differentiation of fibroblast-like cells from RA bone marrow CD34+ cells. Since MTX, but not BUC, has been previously shown to influence on type A synoviocytes, the data provide rationale of combination of BUC and MTX in the treatment of RA.

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1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by hyperplasia of synovial lining cells [1]. Although the dysregulated proliferation of synoviocytes has been suggested to play a pivotal role in synovial hyperplasia [2], it was found that rheumatoid synovium rarely showed evidence of mitosis and that only 4% of rheumatoid synovial cells showed uptake of thymidine [3]. Previous studies have suggested that abnormal myelopoiesis in the bone marrow might play an important role in the pathogenesis of RA [4]. Thus, it has been demonstrated that the generation of CD14 (+) monocyte lineage cells (type A synoviocyte-like cells) from the bone marrow is accelerated in RA patients [5]. In addition, recent studies have disclosed that bone marrow CD34+ cells have enhanced capacity to differentiate into cells with characteristic features of fibroblast-like synoviocytes (type B synoviocytes) in RA [6]. These results raise the possibility that the synovial hyperplasia in RA might be a result of continuous recruitment of bone marrow-derived cells into the synovium [7].

Disease-modifying antirheumatic drugs (DMARDs) have been the mainstay of treatment of RA in recent years. Methotrexate (MTX) clearly has reproducible biological effects on disease activity of RA [8–10] which are sustained over prolonged intervals [11,12]. Although many efforts have been made to explore the mechanisms of action of DMARDs, predominantly on immunocompetent cells, endothelial cells, and synovial cells [13,14], the basis for the efficacy of DMARDs has not been fully elucidated. Recently it has been proposed that DMARDs may work by inhibiting myelopoiesis by supplying fewer inflammatory cells in the inflamed joints [4]. In fact, we have shown that gold sodium thiomalate (GST) as well as MTX inhibits the generation of CD14 (+) cells (type A synoviocyte-like cells) from the bone marrow progenitor cells of RA patients confirming that bone marrow progenitor cells are one of the targets of DMARDs [15,16]. However, the influence of DMARDs, including MTX, on the generation of type B synoviocyte-like cells has not been delineated. The current study therefore examined the effect of MTX and bucillamine (BUC), another potent DMARD, on the *in vitro* generation of type B

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doi:10.1016/j.intimp.2008.10.007

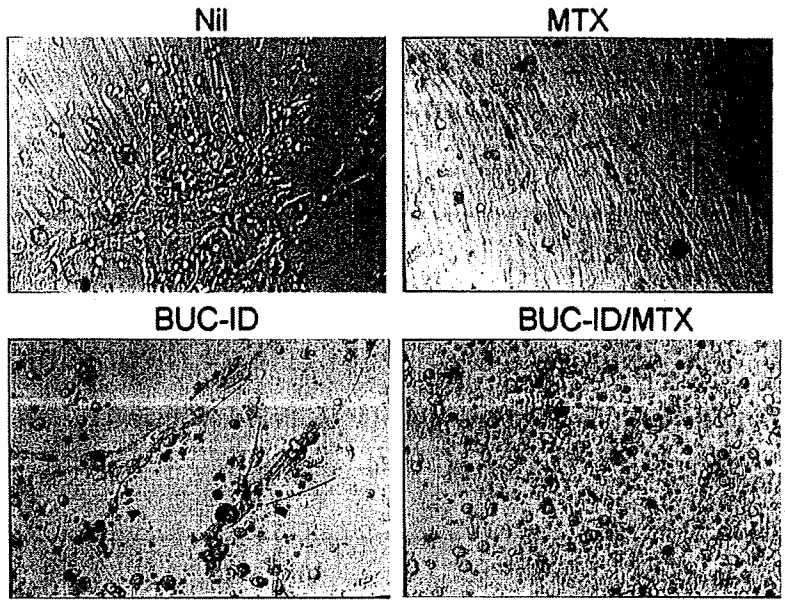

Fig. 1. Morphological changes of CD34+ cells cultured in the presence of SCF, GM-CSF and TNF-α. CD34+ cells from bone marrow of RA patients (1×10^5/well) were cultured in the presence of SCF (10 ng/ml), GM-CSF (1 ng/ml) and TNF-α (10 ng/ml). MTX (methotrexate) (20 nM) or BUC-ID (intramolecular disulfide form of bucillamine) (3 μM) was added where indicated (nil: no addition). After 4 weeks, the morphological changes were determined by phase-contrast microscopy. Original magnification ×50. The data of a representative experiment are shown.

synoviocyte-like cells from bone marrow CD34+ progenitor cells of RA patients.

2. Patients and methods

2.1. Patients

Iliac bone marrow samples were obtained during joint operations from 8 female patients with active RA (mean age 56.6 years, range 43 to 72 years), who gave informed consent. All 8 RA patients fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) 1987 revised criteria for the disease [17]. None of the 8 patients were receiving BUC, whereas 5 of the 8 patients were receiving MTX at the study.

2.2. Reagents

MTX was purchased from Sigma Chemical Co. (St. Louis, MO), and intramolecular disulfide form of BUC (BUC-ID) was a gift of Santen Pharmaceutical Co. (Osaka, Japan). Recombinant human granulocyte macrophage-colony stimulating factor (GM-CSF), stem cells factor (SCF), and tumor necrosis factor-α (TNF-α) were purchased from Pepro Tech EC (London, United Kingdom).

2.3. Culture medium

All cultures were carried out in medium RPMI 1640 (Life Technologies, Grand Island, NY) supplemented with penicillin G (100 U/ml), streptomycin (10 μg/ml), L-glutamine (0.3 mg/ml), and 10% fetal bovine serum (Life Technologies).

2.4. Preparation and culture of bone marrow cells

Heparinized bone marrow aspirates were obtained from the posterior iliac bone. Mononuclear cells were isolated by centrifugation of heparinized bone marrow aspirates over sodium diatrizoate-Ficoll gradients (Histopaque; Sigma Chemical Co., St Louis, MO). CD34+ cells were purified from the bone marrow mononuclear cells by positive selection with magnetic beads (CD34 progenitor cells selection system; Dynal, Oslo, Norway). The cells thus prepared were >95% CD34+ cells and <0.5% CD19+ B cells, as previously described [6]. CD34+ cells were incubated in a 24-well microtiter plate with flat-bottomed wells (No. 3524; Costar, Cambridge, MA) (1×10^5/well) with SCF (10 ng/mL), GM-CSF (1 ng/mL) and TNF-α (10 ng/mL) in the presence or absence of pharmacologically relevant concentrations of BUC-ID (3 μM) [18] and MTX (20 nM) [19]. Preliminary experiments disclosed that MTX as well as BUC-ID in the culture medium was stable after 28 days of incubation, with residual concentrations of approximately 90%. After incubation for 28 days, the cells were observed under phase-contrast microscopy and the culture supernatants were assayed

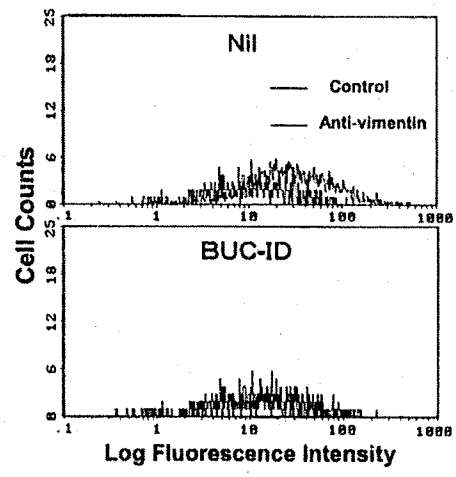

Fig. 2. Expression of vimentin in CD34+ cells cultured in the presence of SCF, GM-CSF and TNF-α. CD34+ cells from bone marrow of RA patients (1×10^5/well) were cultured in the presence of SCF (10 ng/ml), GM-CSF (1 ng/ml) and TNF-α (10 ng/ml). BUC-ID (intramolecular disulfide form of bucillamine) (3 μM) was added where indicated (nil: no addition). After 4 weeks, the cells were harvested and were examined by flow cytometry for the expression of vimentin as described in Materials and Methods.

for matrix metalloproteinase-1 (MMP-1) with the Biotrak human MMP-1 enzyme-linked immunosorbent assay system (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom) and for vascular endothelial growth factor (VEGF) with the human VEGF immunoassay kit (Bio source International, Camarillo, CA). The concentrations of β_2-microglobulin (β_2MG) were determined by a sandwich ELISA as previously described [20].

2.5. Immunofluorescence staining and analysis

Cultured CD34$^+$ cells were fixed with 1% paraformaldehyde in PBS for 5 min at room temperature and were permeabilized in PBS (pH 7.2) containing 2% normal human AB serum, 0.1% sodium azide, and 0.1% saponin (Sigma), followed by staining with fluorescein isothiocyanate (FITC)-conjugated anti-human vimentin mAb (mouse IgG2a; Progen Biotechnik GMBH, Heidelberg, Germany), or FITC-conjugated isotype-matched control mAb (Dako, Glostrup, Denmark). The cells were then analyzed using an EPICS XL flow cytometer (Coulter, Hialeah, FL), as previously described [6].

3. Results

Fig. 1 depicts the representative patterns of the generation of fibroblast-like cells from RA bone marrow CD34+ cells upon stimulation with SCF, GM-CSF and TNF-α for 28 days in the presence or absence of pharmacologically attainable concentrations of MTX and BUC-ID. It is clear that the generation of fibroblast-like cells was suppressed by BUC-ID, but not by MTX. As can be seen in Fig. 2, the expression of vimentin in bone marrow CD34+ cells was markedly suppressed by BUC-ID, confirming that BUC-ID inhibits the generation of fibroblast-like cells. Table 1 compares the effects of MTX and BUC-ID on the generation of fibroblast-like cells from bone marrow CD34+ cells in 8 RA patients. The degree of the generation of fibroblast-like cells was scored as 1 (trace, 0–5%), 2 (mild, 5–30%), 3 (moderate, 30–50%), 4 (strong, >50%), 5 (very strong, with the formation of a cluster or a pile), depending on the observation from two view fields at ×50 magnification. The generation of fibroblast-like cells from RA bone marrow CD34+ cells was significantly suppressed by BUC-ID, but not by MTX. Although 5 of the 8 RA patients were receiving MTX at the study, the in vitro effects of MTX or BUC-ID on the generation of fibroblast like cells were not significantly different irrespective of the administration of MTX.

Table 1
Effects of methotrexate and bucillamine on the generation of fibroblast-like cells from CD34+ cells stimulated with SCF, GM-CSF, and TNF-α[1]

Patients		Generation of fibroblast-like cells[1]			
Age/gender	DMARDs#	Nil	BUC-ID	MTX	BUC-ID/MTX
60/F	MTX	2	1	1	1
43/F	MTX	5	1	1	1
51/F	MTX	5	2	2	2
72/F	SASP+DPC	4	1	3	1
51/F	MTX	5	5	5	5
46/F	MTX+SASP	4	2	5	1
64/F	None	2	1	4	3
66/F	None	4	4	5	5
	(mean±SD)	(3.875±1.166)	(2.125±1.452)*	(3.25±1.639)	(2.375±1.653)*

[1]CD34+ cells from bone marrow of 8 RA patients (1×10^5/well) were cultured in the presence of SCF (10 ng/ml), GM-CSF (1 ng/ml) and TNF-α (10 ng/ml). MTX (methotrexate) (20 nM) or BUC-ID (intramolecular disulfide form of bucillamine) (3 μM) was added where indicated (nil: no addition). After 4 weeks, the generation of fibroblast-like cells was evaluated and scored as 1 (trace), 2 (mild), 3 (moderate), 4 (strong), or 5 (very strong). Statistical significance was determined with Wilcoxon signed rank test.
#At the study, 6 of the 8 RA patients were receiving DMARDs, including MTX, sulfasalazine (SASP), d-penicillamine (DPC).
*Significant at $p<0.05$.

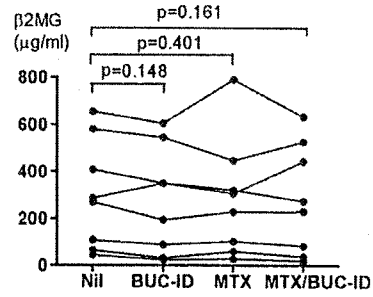

Fig. 3. Concentration of β_2MG in the culture supernatants of CD34+ cells stimulated with SCF, GM-CSF, and TNF-α. CD34+ cells from bone marrow of 8 patients (1×10^5/well) were cultured in the presence of SCF (10 ng/ml), GM-CSF (1 ng/ml) and TNF-α (10 ng/ml). MTX (methotrexate) (20 nM) or BUC-ID (intramolecular disulfide form of bucillamine) (3 μM) was added where indicated (nil: no addition). After 4 weeks, the culture supernatants were harvested and analyzed for β_2MG content by enzyme linked immunosorbent assay. Statistical significance was determined with Wilcoxon signed rank test.

Previous studies disclosed that β_2MG is produced by a number of cell types, including lymphocytes, myeloid cells, and tumor cells [21–23]. The production of β_2MG generally correlates with cell proliferation [21–23]. In fact, the levels of β_2MG in the culture supernatants paralleled the viable cell counts of bone marrow CD34+ cells stimulated with SCF, GM-CSF and TNF-α [24]. To explore whether MTX and BUC-ID might influence the cell out-growth, the effects of these drugs on the production of β_2MG were examined. As shown in Fig. 3, either MTX or BUC-ID did not significantly influence the production of β_2MG by bone marrow CD34+ cells.

Previous studies suggested that the fibroblast-like cells have capacities to produce MMP-1 and VEGF, a feature that is unique to type B synoviocytes [25]. It has been well known that MMP-1 plays an important role in the destruction of cartilage and bone in RA [6]. In addition, VEGF is a key cytokine for angiogenesis, which plays a pivotal role in synovial hyperplasia in RA [26]. We next examined the effects of MTX and BUC-ID on the production of MMP-1 in cultures of RA bone marrow CD34+ cells stimulated with SCF, GM-CSF and TNF-α for 28 days. As can be seen in Figs. 4 and 5, the production of MMP-1 as well as that of VEGF was significantly suppressed by BUD-ID, but not

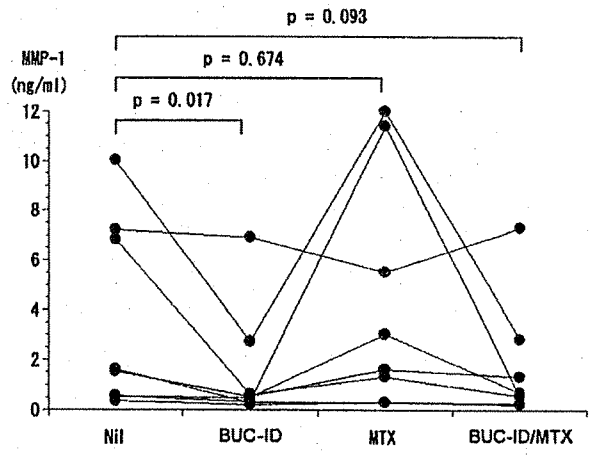

Fig. 4. Concentration of MMP-1 in the culture supernatants of CD34+ cells stimulated with SCF, GM-CSF, and TNF-α. CD34+ cells from bone marrow of 8 patients (1×10^5/well) were cultured in the presence of SCF (10 ng/ml), GM-CSF (1 ng/ml) and TNF-α (10 ng/ml). MTX (methotrexate) (20 nM) or BUC-ID (intramolecular disulfide form of bucillamine) (3 μM) was added where indicated (nil: no addition). After 4 weeks, the culture supernatants were harvested and analyzed for MMP-1 content by enzyme linked immunosorbent assay. Statistical significance was determined with Wilcoxon signed rank test.

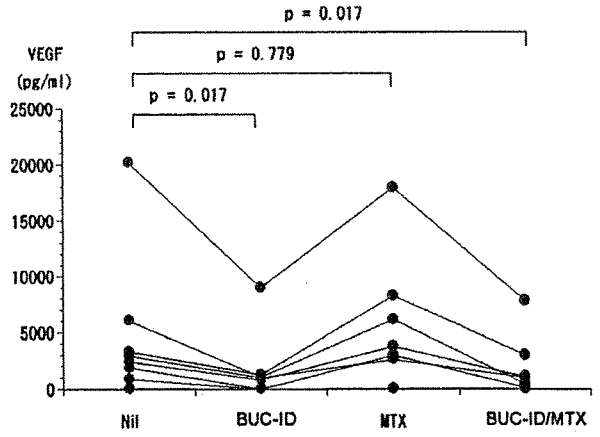

Fig. 5. Concentrations of VEGF in the culture supernatants of CD34+ cells stimulated with SCF, GM-CSF, and TNF-α. CD34+ cells from bone marrow of 8 RA patients (1×10^5/well) were cultured in the presence of SCF (10 ng/ml), GM-CSF (1 ng/ml) and TNF-α (10 ng/ml). MTX (methotrexate) (20 nM) or BUC-ID (intramolecular disulfide form of bucillamine) (3 μM) was added where indicated (nil: no addition). After 4 weeks, the culture supernatants were harvested and analyzed for VEGF content by enzyme-linked immunosorbent assay. Statistical significance was determined with Wilcoxon signed rank test.

by MTX. These results might support the conclusion that BUC-ID, but not MTX, suppress the generation of type B synoviocyte-like cells from bone marrow CD34+ cells in RA.

4. Discussion

Type B synoviocytes, which are also called fibroblast-like synoviocytes, have the morphological appearance of fibroblasts as well as the capacity to produce a variety of factors, including cytokines and matrix metalloproteinases (MMPs), that lead to the destruction of joints [25]. We have recently demonstrated that bone marrow CD34+ cells from RA patients have abnormal capacities to respond to TNF-α and to differentiate into fibroblast-like cells producing MMP-1 [6]. The results in the current studies have disclosed that BUC-ID, but not MTX, significantly suppressed the generation of fibroblast-like cells from RA bone marrow CD34+ cells at their pharmacologically relevant concentrations. Of note, since mesenchymal stem cells have been recently implicated in the generation of the fibroblast-like cells in RA [27], the use of CD34+/CD45− cells would help delineate which bone marrow population is responsible for the production of the fibroblast-like cells. Further studies are required to delineate this point.

MTX has now become internationally the first choice among DMARDs for active RA [8,9]. Previous studies showed that MTX at as little as 5 nM suppressed the growth of differentiated monocytic myeloid cell line THP-1 [28]. In addition, it was found that MTX at 20 nM suppressed the proliferation of human monocytic cells line U937 cells as well as the production of IL-1β by RA bone marrow mononuclear cells [29]. Taken together, these data suggest that MTX might inhibit the generation of type A synoviocytes and their function [16]. On the other hand, BUC-ID at 3μM did not suppress the generation of CD14+ cells from RA bone marrow CD14− cells, suggesting that BUC-ID might not affect the generation of type A synoviocytes and their function in RA [15].

Of note, in the present study, BUC-ID at 3 μM significantly suppressed the generation of fibroblast-like cells irrespective of the presence of MTX in cultures of RA bone marrow CD34+ cells stimulated with SCF, GM-CSF and TNF-α. In addition, BUC-ID inhibited the expression of vimentin, a fibroblastic-specific marker, in cultured bone marrow CD34+ cells. Finally, BUC-ID also suppressed the production of VEGF and MMP-1 in these cultures. These results suggest that BUC-ID might have suppressive influences on the generation of type B synoviocytes and their function in RA. On the other hand, MTX at 20 nM did not suppress either the generation of fibroblast-like cells from RA bone marrow CD34+ cells or the production of MMP-1 and VEGF in these cultures, suggesting that MTX might not affect the generation of type B synoviocytes and their function in RA.

Recent double-blinded, randomized controlled studies have provided evidence that the combination therapy with MTX and BUC resulted in significantly higher clinical efficacy than either monotherapy [30]. Thus, the patients treated with the combination of MTX and BUC showed significantly higher ACR 20 response rate (79.2%) than those treated with MTX alone (43.5%), or with BUC alone (45.8%) in a 96-week trial [30]. As mentioned above, the data in previous and current studies suggest that the targets of MTX and BUC might be different. Thus, MTX appears to suppress the generation and the function of type A synoviocytes, whereas BUC-ID seems to inhibit the generation and the function of type B synoviocytes. Since both type A synoviocytes and type B synoviocytes play an important role in the pathogenesis of RA, these results might account for the clinical efficacy of the combination of MTX and BUC.

It has been shown that MTX effects cellular metabolism at several different steps by inhibition of dihydrofolate reductase, thymidylate synthase, and aminoimidazole carboxamide ribonucleotide transformylase [31]. By contrast, the mechanisms of action of BUD-ID still remain unclear. On the other hand, we have recently revealed that the activation of NFkB1 plays a pivotal role in the abnormal capacity of RA bone marrow CD34+ cells to differentiate into fibroblast-like cells and to produce MMP-1 and VEGF [24]. In this regard, previous studies disclosed that high concentrations of BUC (1 mM) directly inhibited the activation of NFkB in murine system [32]. Since conversion between BUC and BUC-ID might take place in vitro as well as in vitro, it is possible that BUC-ID might also be involved in the inhibition of the activation of NFkB. It is therefore likely that the inhibitory influences of BUC-ID on the generation of fibroblast-like cells from RA bone marrow CD34+ cells might be a result of inhibition of the activation of NFkB. Further studies would be required to explore whether BUC-ID might inhibit the activation of NFkB at pharmacologically relevant concentrations.

Acknowledgment

This work was supported by a grant from Santen Pharmaceutical Co., Ltd., Osaka, Japan.

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Mod Rheumatol (2009) 19:180–186
DOI 10.1007/s10165-008-0147-8

ORIGINAL ARTICLE

Treatment of juxta-articular intraosseous cystic lesions in rheumatoid arthritis patients with interconnected porous calcium hydroxyapatite ceramic

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Received: 16 June 2008 / Accepted: 18 November 2008 / Published online: 27 January 2009
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Abstract In patients with rheumatoid arthritis (RA), juxta-articular intraosseous cystic lesions may cause spontaneous pathological fractures. The outcome of curettage and the packing of such lesions with interconnected porous calcium hydroxyapatite ceramic (IP-CHA) was investigated. Twelve lesions were treated in ten RA patients (three men and seven women with a mean age of 59 years). Ten lesions were associated with impending pathological fracture involving the articular surface. In all patients, curettage and packing of the bone cavity with IP-CHA were done. Assessment was based on final radiographs obtained an average of 30 months after surgery (range 10–47 months). Absorption of the implanted IP-CHA, expansion of the lesion, implant incorporation into host bone, and postoperative fractures were investigated. At final follow-up, there was no absorption of the implanted IP-CHA in any of the lesions. Expansion of the radiolucent area was only noted in one lesion. Seven of the other 11 lesions showed major incorporation of IP-CHA into host bone, while minor incorporation was seen in four lesions. There were no postoperative fractures. In conclusion, curettage and packing with IP-CHA is a feasible method of preventing pathological fracture due to juxta-articular intraosseous cystic lesions in RA patients.

Keywords Interconnected porous calcium hydroxyapatite ceramic · Juxta-articular intraosseous cystic lesions · Rheumatoid arthritis · Surgical treatment

Electronic supplementary material The online version of this article (doi:10.1007/s10165-008-0147-8) contains supplementary material, which is available to authorized users.

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Introduction

Juxta-articular intraosseous cystic lesions in patients with rheumatoid arthritis (RA) have been variously termed synovial cysts [1], subchondral cysts [2], subarticular pseudocysts [3], or geodes [4]. These lesions are often found during the course of RA, but aggressive surgical treatment is not usually performed because the patient has no symptoms unless spontaneous or traumatic intra-articular fracture occurs. Once fracture occurs, however, joint destruction progresses rapidly due to mechanical stress rather than disease-related inflammation, and daily activities can be severely affected [5–8]. It is important to maintain the subchondral bone of RA patients in good condition, not only to prevent pathological fractures but also because of the possible need to perform arthroplasty in the future.

We have treated juxta-articular intraosseous cystic lesions in RA patients by curettage and packing with a hydroxyapatite filler (interconnected porous calcium hydroxyapatite ceramic, IP-CHA) in order to prevent subchondral fractures. In our experience, IP-CHA undergoes

extensive incorporation into host bone more rapidly than other types of porous calcium hydroxyapatite ceramic [9]. The purpose of the present study was to evaluate the preliminary results obtained with this treatment.

Materials and methods

Between September 2003 and March 2005, we treated 12 juxta-articular cystic lesions in ten RA patients (Table 1). Ten of the 12 lesions were associated with a risk of impending intra-articular fracture, judging from the fragile appearance of the subchondral bone on radiographs. The other two lesions were treated as an additional procedure during synovectomy and matched ulnar resection, respectively. All patients fulfilled the American Rheumatism Association diagnostic criteria for RA [10]. Three patients were men and seven were women, with an average age of 59 years (range 49–72 years) at the time of operation. The location and size of the lesions were determined by examination of preoperative anteroposterior plain X-ray films. Eight lesions were located in the distal radius (four involved most of the subarticular surface, two were located in the radial styloid process, and the remaining two were at the center of the subarticular bone), while one lesion was located in the head of the ulna, one in the center of the proximal tibial condyle, one in the medial malleolus of the tibia, and the lateral malleolus of the fibula. The lesions were classified into three groups on the basis of the transverse diameter: large lesions had a diameter of greater than two-thirds of the articular surface ($n = 6$), medium lesions had a diameter of one-third to two-thirds of the articular surface ($n = 4$), and small lesions had a diameter of less than one-third ($n = 2$). Disease activity was assessed preoperatively from the C-reactive protein (CRP) level and the tenderness and swelling of the involved joint. The Steinbrocker functional class and stage [11] and the Larsen grade [12] of the joint were also assessed. Each patient's medications were recorded (Table 2).

Operative technique

Surgery was performed under regional or general anesthesia. A pneumatic tourniquet was applied. Under fluoroscopic guidance as needed, each juxta-articular lesion was exposed via an extra-articular approach. For lesions of the distal radius, the second or fourth extensor retinaculum was opened through a dorsal skin incision about 2–3 cm long. For lesions of the knee, ankle, and ulna, a skin incision was made just over the target. A small window (about 8 mm square) was made in the wall of the lesion, taking care to preserve the periosteum of the resected bone. After performing intralesional curettage and complete resection of the capsule of the cyst, the residual bone defect was filled with blocks and granules of sterilized IP-CHA (Stryker Co., Tokyo, Japan). Then the small bone section was replaced in order to close the cortical window. After surgery, a splint was not applied and range-of-movement exercises were commenced immediately. Together with the above procedure, synovectomy of the wrist and knee joint was also done in two patients who had wrist synovitis (case 2) and a huge synovial cyst (case 9), respectively. Matched ulnar head resection was also performed in one patient (case 5) who presented with disability of the distal radioulnar joint.

Table 1 Details of the 12 lesions

Case no.	Age	Gender	Location	Size (mm)	Follow-up (months)	Expansion	Absorption	Incorporation (grade)	Combined operation
1	55	M	R distal radius	35 × 20 (large)	42	–	–	2	
			L distal radius	35 × 12 (large)		–	–	3	
			Distal ulna	16 × 9 (large)		–	–	3	
2	52	F	Proximal tibia	50 × 32 (medium)	10	–	–	2	#1
3	61	F	Distal fibula	33 × 21 (large)	40	–	–	3	
4	53	F	Distal radius	19 × 15 (medium)	47	–	–	3	
5	72	F	Distal radius	11 × 10 (small)	22	–	–	3	#2
6	63	M	Distal radius	25 × 12 (medium)	37	–	–	3	
7	49	F	Distal radius	35 × 20 (large)	16	+	–	–	
8	59	M	Distal radius	16 × 9 (medium)	19	–	–	2	
9	55	F	Distal radius	7 × 4 (small)	36	–	–	3	#1
10	71	F	Distal tibia	29 × 17 (large)	29	–	–	2	

Expansion expansion of the radiolucent area, *Absorption* absorption of implanted IP-CHA, *Combined operation* other procedures performed simultaneously, *#1* synovectomy, *#2* matched ulnar resection

Table 2 Details of the ten patients

Case no.	Preoperative CRP (mg/dl)	Location	Class	Stage	Larsen grade	Local tenderness	Local swelling	Medications (daily doses except for MTX)
1	0.9	R distal radius	3	4	4	Mild	Mild	Predonine 10 mg, MTX 6 mg
		L distal radius			4	Mild	Mild	
		Distal ulna			4	Mild	Mild	
2	2.9	Proximal tibia	2	3	2	Moderate	Severe	Predonine 10 mg, MTX 6 mg, bucillamine 300 mg
3	0.2	Distal fibula	2	3	4	Non	Non	MTX 4 mg, metronidazole, salazosulfapyridine 1,000 mg
4	0.2	Distal radius	2	3	4	Mild	Mild	Predonine 7.5 mg, MTX 6 mg
5	1.3	Distal radius	3	2	4	Moderate	Moderate	Predonine 5 mg, salazosulfapyridine 1,000 mg
6	0.9	Distal radius	2	3	3	Mild	Moderate	Predonine 5 mg, bucillamine 200 mg
7	2.5	Distal radius	3	3	5	Moderate	Moderate	Predonine 10 mg
8	0.8	Distal radius	2	3	5	Mild	Mild	Salazosulfapyridine 1,000 mg
9	0.4	Distal radius	1	2	3	Moderate	Moderate	Predonine 1 mg, salazosulfapyridine 1,000 mg
10	1.2	Distal tibia	2	4	2	Non	Non	Salazosulfapyridine 1,000 mg

Class Steinbrocker functional class, *Stage* Steinbrocker stage, *MTX* methotrexate (weekly doses)

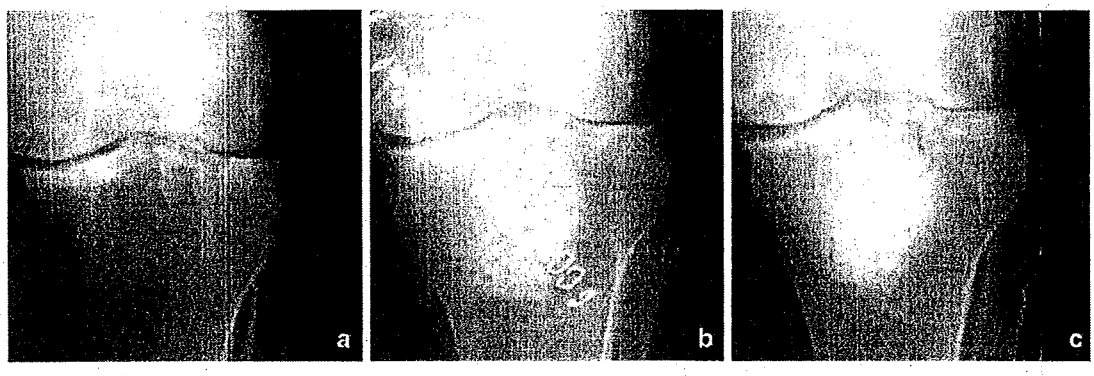

Fig. 1 Case 2: **a** preoperative radiograph reveals a large radiolucent area in the proximal tibia. **b** Radiograph obtained just after surgery. **c** At 10 months after surgery, there is no absorption of IP-CHA or expansion of the cystic lesion

Radiological assessment

Radiological assessment was performed by comparing radiographs obtained just after surgery with those obtained at final follow-up at an average of 30 months (range 10–47 months). The following four points were assessed: absorption of the implanted IP-CHA, expansion of the cystic lesion, incorporation of the implanted IP-CHA into host bone, and occurrence of postoperative fracture. The extent of incorporation of the implanted IP-CHA by host bone was graded according to the previously reported method [9]. In brief, Grade 1 was no incorporation, Grade 2 was minor incorporation (a slight increase in the density of the implanted IP-CHA granules and partial disappearance of the radiolucent lines between implant and host bone), and Grade 3 was major incorporation (a marked increase of density and/or disappearance of the spaces between IP-CHA granules).

Clinical assessment

Complications such as fracture, infection, and joint contracture related to surgery were assessed by clinical review.

Results

Absorption of the implanted IP-CHA did not occur in any patient and there were no postoperative fractures. Eleven of the 12 lesions showed no expansion at final follow-up. In these 11 lesions, the density of the implanted IP-CHA increased over time, and the granules appeared to become fused and incorporated into the surrounding host bone. Four of the 11 lesions showed grade 2 incorporation and seven lesions showed grade 3 incorporation at final follow-up (Figs. 1, 2). One patient (case 7) had poorly controlled RA due to concomitant hepatic and pancreatic dysfunction,

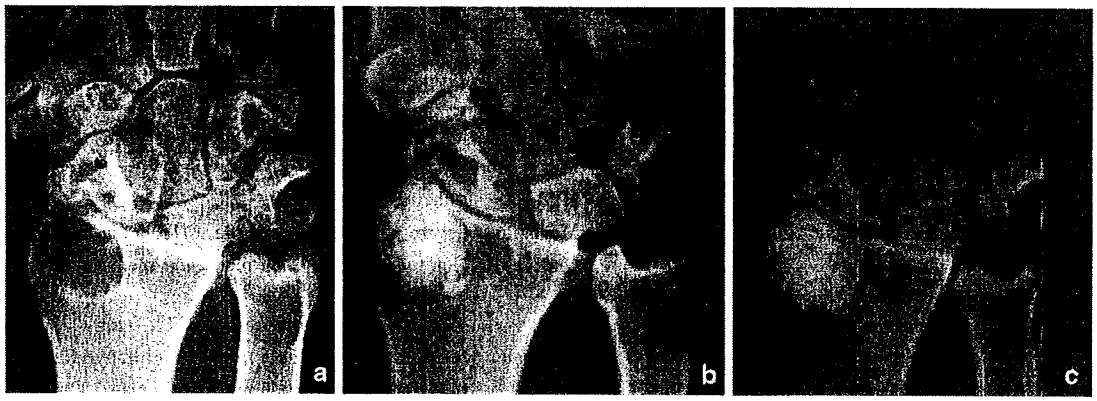

Fig. 2 Case 6: **a** preoperative radiograph reveals a large radiolucent area in the distal radius. **b** Appearance just after surgery. **c** At 37 months after surgery, there is no absorption of IP-CHA or expansion of the cystic lesion

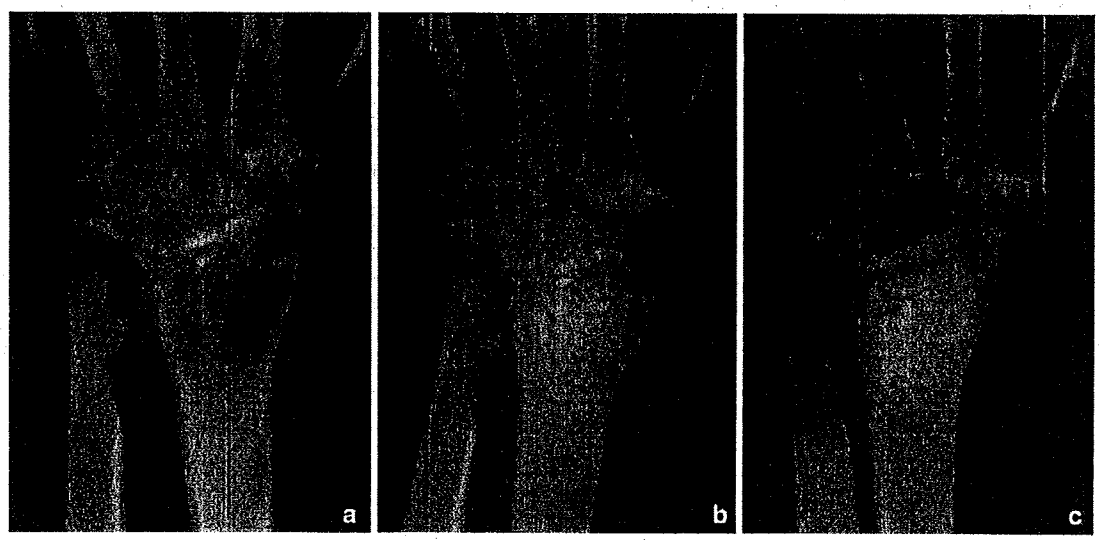

Fig. 3 Case 7: **a** preoperative radiograph reveals a large radiolucent area in the distal radius. **b** Appearance just after the operation. Curettage and IP-CHA implantation are inadequate. **c** At 16 months after surgery, there is expansion of the cystic lesion, but no reabsorption of implanted IP-CHA

and expansion of the cystic lesion occurred with appearance of a radiolucent zone around the implanted IP-CHA at final follow-up (Fig. 3).

Before surgery, cases 5 and 9 had pain and swelling of the wrist. Their pain and swelling improved after synovectomy in addition to curettage and packing of the lesions. In case 2 (Fig. 1), preoperative knee joint pain was improved after curettage of a tibial cystic lesion and packing with IP-CHA, as well as synovectomy of the knee joint. The range of joint motion did not change significantly after surgery. There were no complications related to implanting IP-CHA, such as excessive postoperative drainage, erythema, or wound infection.

Discussion

Juxta-articular intraosseous cystic lesions are commonly found during the course of RA, and these lesions can cause spontaneous pathological fractures that result in extensive joint destruction [5–8]. Nakagawa et al. reported two cases of giant geodes involving the olecranon process. They performed surgery for an associated pathological fracture in one patient and treated the other patient prophylactically with an autologous iliac bone graft to prevent fracture [5]. Lowthian et al. reported a patient who had multiple pathological fractures of the phalanges due to cystic lesions that led to residual instability of the

fingers despite surgical treatment [6]. Wordsworth et al. reported a patient with a pathological fracture of the proximal ulna that was initially treated conservatively but failed to unite, so that bone grafting was subsequently performed [7]. Rappoport et al. reported three patients with chronic RA who developed pathological fractures of the olecranon process due to erosions or cysts [8]. Although intraosseous cystic lesions often occur in patients with active RA and wrist joint involvement, it is difficult to detect such lesions associated with fracture of the distal radius by either clinical or radiographic assessment. One reason might be that intermittent radiological evaluation does not allow detection of pathological fracture of the subchondral bone associated with an intraosseous cystic lesion. The second reason might be that concomitant pathological changes, including erosions, destruction of cartilage, and subluxation of the radiocarpal joint, make it difficult to detect an intraosseous cystic lesion associated with fracture of the distal radius. In one of our patients, joint collapse was associated with the cystic lesion on radiographs. Figure 4a shows a double contour indicating a fracture adjacent to this patient's intraosseous cystic lesion, while Fig. 4b displays rapid progression of joint destruction after six months. Once joint destruction has occurred (as in Fig. 4b), it is quite difficult to detect the presence of a cystic lesion near the radiocarpal joint. The findings in this patient suggest that prophylactic surgical treatment of juxta-articular intraosseous cystic lesions in RA patients could be useful to prevent impending fracture, as is the case with benign bone tumors like solitary bone cyst or giant cell tumor of bone. However, surgery is not often performed in RA patients for the following reasons (among others). First, a juxta-articular intraosseous cystic lesion causes no symptoms or inconvenience itself. Second, autologous bone grafting is associated with donor site morbidity [13], and there are limitations on grafting because of the possible need for multiple operations in patients with RA. Third, an artificial bone substitute with excellent bone conduction properties was not available in the past. Conventional hydroxyapatite implants achieve little bone ingrowth into the deeper regions because there are few interpore connections allowing bone marrow cells to infiltrate into the implant, so a hydroxyapatite implant does not gain sufficient mechanical strength. On the other hand, IP-CHA has demonstrated excellent osteoconductivity in animal models as well as in clinical trials [9, 14]. IP-CHA has a high porosity (75%), and the pores show uniform connections with each other like the pores in cancellous bone. The majority of the pores are approximately 100–200 μm in diameter with interconnections that are about 40 μm in diameter, and the compression strength of IP-CHA is about 10 MPa [14]. These structural differences from conventional hydroxyapatite confer excellent bone conduction, so that host bone ingrowth is sufficient to achieve adequate mechanical strength without autologous bone grafting. On the other hand, Suzuki et al. reported a patient with a giant geode in the tibial condyle that was successfully treated with calcium phosphate cement (CPC) [15]. Although the use of CPC provides initial high mechanical strength and incorporation of the implant surface into host bone may occur, a CPC block has no pores. IP-CHA provides sufficient mechanical strength after new bone ingrowth occurs due to its cancellous

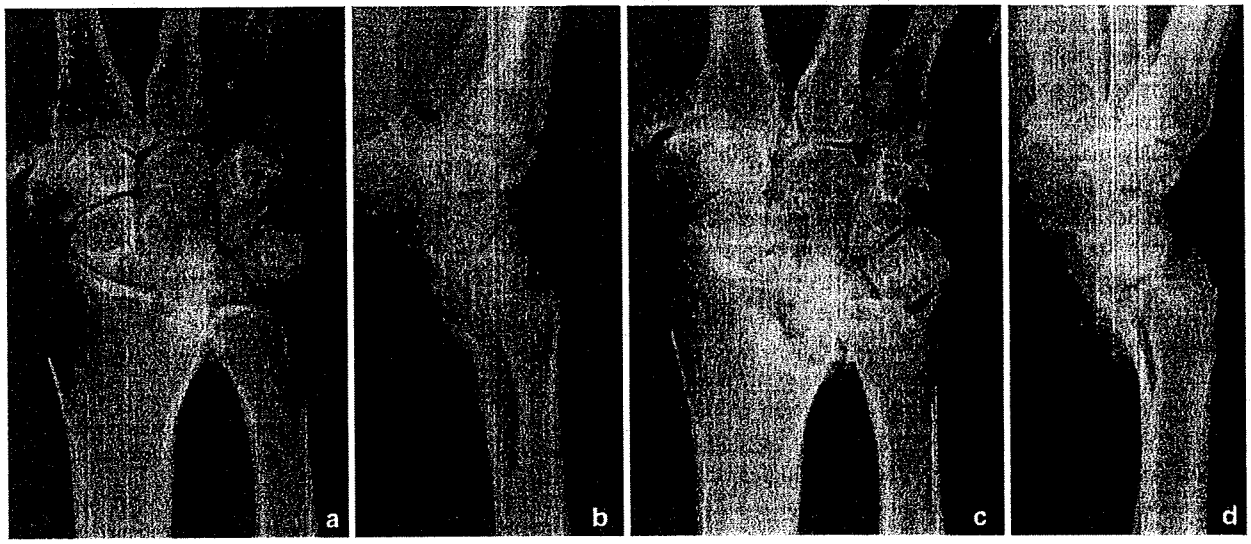

Fig. 4 A 54-year-old man: **a**, **b** there is a pathological fracture and cystic lesion of the lunate fossa in the distal radius. **c**, **d** After 6 months, joint destruction shows marked progression

structure, and it might also show more physiological resistance to mechanical stress. We aimed to utilize these properties of IP-CHA to improve mechanical strength in the treatment of intraosseous cystic lesions, and achieved good radiological results in the present series. We also found that IP-CHA is easy to handle and there is little risk of leakage into adjacent joints.

In patients with RA, the bone adjacent to involved joints develops juxta-articular osteoporosis, erosions, and intraosseous cystic lesions, with these changes being considered to be due to an increase of osteoclastic bone resorption [16–19] secondary to local overproduction of bone-resorbing cytokines. The present study revealed that IP-CHA was resistant to absorption, even in the pathological state of RA for at least 47 months after surgery. This property of IP-CHA makes it suitable for treating RA because a more biodegradable material could also be remodeled into bone but might then be affected by juxta-articular osteoporosis or erosive changes.

One of our patients had severe RA that could not be controlled due to concomitant hepatic and pancreatic dysfunction. The CRP level of this patient remained above 2.0 mg/dl before and after surgery, and expansion of the cystic lesion occurred despite surgical treatment. This case suggests that curettage and implantation of IP-CHA will not suppress local disease activity and prevent the expansion of a lesion if the patient has poorly controlled RA. Recent advances in the treatment of RA with the advent of biological agents have made it possible to prevent the progression of joint destruction [20, 21]. Accordingly, the expansion of cystic lesions may also be prevented by controlling disease activity with more effective drugs. Such advances in medical therapy may allow curettage and packing with IP-CHA to become a better treatment for juxta-articular intraosseous cystic lesions.

In patients with RA, the goals of surgical treatment include relief of pain and improvement of joint function. Procedures such as synovectomy, arthrodesis, and resection arthroplasty mainly target pain relief. Implant arthroplasty targets both pain relief and functional improvement, while tendon transfer and tendon grafting are procedures that aim to improve function. The present procedure represents a new category of surgical treatment for RA that is indicated to prevent impending articular fracture due to a large intraosseous cystic lesion.

In RA patients, joint destruction is a process that involves several events, including destruction of the surface cartilage, resorption of mineralized cartilage, and erosion of subchondral bone [22]. Pathological fracture associated with a juxta-articular intraosseous cystic lesion can also contribute to joint destruction, but not all cystic lesions will cause fractures. The size and location of a lesion, as well as juxta-articular osteoporosis, marginal sclerosis, systemic disease activity, and the local inflammatory response might all influence the risk of fracture. To adequately decide the indications for this procedure, a method for assessing the risk of impending fracture associated with juxta-articular intraosseous cystic lesions is the next issue to be clarified. Recently, we have been evaluating the impending fracture with CT, which shows the size, location and features of a lesion clearly (see Fig. 1 in the "Electronic supplementary material"). Limitations of the present study include the small number of subjects and the lack of a control group. However, these preliminary results are still encouraging and suggest that treatment with IP-CHA may become a useful method for preventing fracture associated with juxta-articular intraosseous cystic lesions in RA patients.

Conflict of interest statement The authors declare that there are no competing financial interests.

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